MCLAB
Molecular Cloning Laboratories

User Manual

Version 1.0
Revision Date: 09/19/2013

Product name: HB101 Competent E. Coli

Cat #: HB-100, HB-196

Description:
High stability, high efficiency, no heat shock necessary. For Research Use Only.

Application:
- Ideal for sub-cloning and scale-up applications
- HB101 strain is a hybrid K12 x B bacterium, containing the recA13 mutation that minimizes recombination and aidsin insert stability. In addition, it carries the hsdS20(rB-mB-) restriction minus genotype which prevents cleavage of cloned DNA by endogenous restriction enzymes. HB101 strain does not support Alpha-Complementation for blue/white screening. Transform efficiency is around 1.0x107 ~ 1.0x109 cfu/μg with pUC18 control DNA.

Recommended storage condition:
This product should be stored at -80°C. Thaw on ice only before use. Do not re-freeze.

Recommended reaction conditions:
- Mix DNA with competent cell
- Let stand on bench for 5 minutes
- Direct plate

Genotype:
F- mcrB mrr hsdS20(rB- mB-) recA13 leuB6 ara-14 proA2 lacY1 galK2 xyl-5 mtl-1 rpsL20(SmR) glnV44 λ-

Protocol:
A stock pUC19 solution (0.1 μg/ml) is provided as a control to determine the transformation efficiency. To obtain maximum efficiency, the experimental DNA must be free of phenol, ethanol, protein and detergents.
1. Thaw competent cells on wet ice. Place required number of autoclaved 1.5-ml microcentrifuge tubes on wet ice.
2. Gently mix cells, then aliquot 50 μl of competent cells into chilled microcentrifuge tubes. (If desired, a 100-μl transformation may be performed.)
3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning them to the -70°C freezer. Do not use liquid nitrogen.
4. To determine transformation efficiency add 5 μl (500 pg) control pUC19 to one tube containing 50 μl competent cells. Move the pipette through the cells while dispensing. Gently tap the tube to mix.
5. Add 1-3 μl (1-10 ng of DNA) of the DNA ligation reaction directly to a second tube containing 50 μl competent cells, moving the pipet through the cells while dispensing. Gently tap the tube to mix.
6. Incubate the cells on ice for 30 minutes.
7. Heat-shock the cells for 20 seconds at 37°C. (If a 100-μl transformation is used, heat-shock for 45 seconds).

Do not shake.
8. Place on ice for 2 minutes.
9. Add 0.95 ml room temperature LB, YT (1) or S.O.C. Medium.
10. Shake at 225 rpm for 1 hour at 37°C for expression (13 × 100 mm glass tubes will hold microcentrifuge tubes in a shaker rack.)
11. After expression, dilute 0.1 ml of the reaction containing the control pUC19 into 0.9 ml medium. Spread 100 µl of the undiluted reaction and the 1:10 dilution on LB or YT plates with 100 µg/ml ampicillin. Vortex briefly before dilution in case cells have settled out.

Notes:

1. Subcloning HB101 Competent Cells may be refrozen. Subsequent freeze-thaw cycles will reduce transformation efficiency approximately 2-fold.
2. Do not use EDTA to stop ligation reactions. Instead, freeze ligations to store for later use. The combination of DNA, ligase, and ligase buffer can inhibit transformation. Optimal results are obtained with T4 Ligase and 5X ligase reaction buffer. If desired, ligations can be concentrated by ethanol precipitation prior to transformation. Dissolve dry pellet in filter sterilized TE buffer. tRNA can be used as a carrier.
3. Subcloning HB101 Competent Cells can support the replication of M13mp vectors. However, HB101 is F- and cannot support plaque formation. The competent cells should be added to top agar after lawn cells, IPTG and Bluo-gal or X-gal have been added. Incubation at 37°C for 1 hour is not required after addition of S.O.C. Medium.